Amendments to the Drawings:

The attached replacement sheet of drawings includes changes to Fig. 9. This sheet, which includes Figs. 9 and 10, replaces the original sheet 6 of 13 of drawings. In Fig. 9, the y-axis is labeled as "Luciferase Activity" in English instead of Japanese.

REMARKS

Upon entry of the foregoing amendments, claims 1-16 and 18-23 will be pending in this application. Claims 1, 2 and 19 are the only independent claims.

Claims 1, 2, 4, 5, 7-10, 12, 15, 16, 18-23 have been amended, without prejudice or disclaimer. Claim 17 is cancelled, without prejudice or disclaimer.

It is respectfully submitted that all claim amendments made herein are fully supported by the claims and specification of the application as originally filed and introduce no new subject matter. For example, the amendment to claim 18 is at least supported by original claim 17, and the amendments to claim 8 and 9 are at least supported by the examples in the specification. Entry of all of the amendments are respectfully requested.

Initially, Applicant is pleased to note that there are no prior art rejections.

Election/Restriction Requirement

The Examiner has made the previous restriction/election of species requirements final. Claims 20-23 are withdrawn from further consideration as being drawn to a nonelected invention. Claims 1-19 are under consideration to the extent they read on the elected species, i.e., SEQ ID NOs: 4 and 5 and their related sequences.

The claims have been amended to cancel the non-elected species, SEQ ID NOs:23 and 24. However, Applicant respectfully requests the Examiner to rejoin SEQ ID NOs:1 and 2 for examination together with SEQ ID NOs: 4 and 5. The Examiner acknowledges that the elected species includes a nucleotide sequence encoding an amino acid sequence exhibiting 95% or more amino acid sequence identity to the amino acid sequence of SEQ ID NO:4. According to the amino acid sequence search results available at the USPTO Public PAIR website, a copy of which is attached to this Amendment as Appendix 1, SEQ ID NO:4 exhibits 96.2% query match with SEQ ID NO:1 (see result 2 in Appendix 1). In addition, according to the nucleic acid sequence search results available at the USPTO Public PAIR website, a copy of which is attached to this Amendment as Appendix 2, SEQ ID NO:2 exhibits 95.9% query match with SEQ ID NO:5 (see result 2 in Appendix 2). Because of the high degree of sequence similarities between the amino acid sequences SEQ ID NOs:1 and 4, and their coding sequences SEQ ID NOs:2 and 5, SEQ ID NOs:1 and 2 should be considered to be within the elected species.

After entry of the claim amendment, claim 1 is directed to an isolated estrogen receptor alpha gene, which encodes the amino acid sequence SEQ ID NO:1 and can have the DNA sequence of SEQ ID NO:2 (see page 60, lines 5-7), and its related sequences; and estrogen receptor alpha 2 gene, which encodes the amino acid sequence SEQ ID NO:4 and can have the DNA sequence of SEQ ID NO:5 (see page 62, lines 11-13), and its related sequences. The estrogen receptor beta gene, which encodes the amino acid sequence SEQ ID NO:23 and can have the DNA sequence of SEQ ID NO:24 (see page 94, last line, to page 95, line 2), and its related sequences, are cancelled, without prejudice or disclaimer.

Accordingly, examination on the merits of claims that read on SEQ ID NOs: 4 and 5, as well as SEQ ID NOs:1 and 2, and their related sequences is respectfully requested.

Objections

The Abstract of the disclosure has been objected to because it allegedly is written in four paragraphs. The abstract is amended to read as a single paragraph in narrative form. Accordingly, withdrawal of the objection to the Abstract is respectfully requested.

Figure 9 is objected to because the X-axis [sic; y-axis] is labeled in Japanese. A replacement sheet of the drawings is submitted. This replacement sheet, which includes Figs. 9 and 10, replaces the original sheet 6 of 13 of drawings. After entry of the replacement sheet, in Fig. 9, the y-axis is labeled as "Luciferase Activity" in English instead of Japanese. Accordingly, withdrawal of the objection to Fig. 9 is respectfully requested.

Claims 4 and 11 have been objected to because of some informalities. Claim 4 has been amended to recite "wherein a promoter is operably linked......" as suggested by the Examiner. Claim 11 has been amended to recite "a mammalian cell" as suggested by the Examiner. Accordingly, withdrawal of the objections to claims 4 and 11 is respectfully requested.

Claims 1, 2 and 19 have been objected to as reciting non-elected species. These claims have been amended to cancel recitations related to SEQ ID NO:23 or 24. As discussed above, SEQ ID NOs:1 and 2, although not explicitly elected, are within the elected species, thus are properly included in the amended claims. Accordingly, withdrawal of the objection to claims 1, 2 and 19 is respectfully requested.

Claim Rejections under 35 USC § 101

Claims 1, 2 and 17-19 are rejected under 35 USC § 101 because the claimed invention is allegedly directed to non-statutory subject matter.

The rejection of claim 17 is moot in view of the cancellation of claim 17 after entry of the claim amendment.

Claims 1, 2, 18 and 19 are amended to recite that the estrogen receptor gene, the DNA or the estrogen receptor is "isolated".

Accordingly, withdrawal of the rejection to claims 1, 2 and 17-19 under 35 USC § 101 is respectfully requested.

Claim Rejections under 35 USC § 112, Second Paragraph

Claims 5, 6, 8-14 and 16-18 are rejected under 35 USC § 112, second paragraph, as being indefinite.

The Examiner asserts that claims 5 and 6 are vague and indefinite, because it is unclear how a viral particle can contain a virus.

Claim 5 is amended to recite that the vector is a "viral vector". The term "viral vector" has a definite meaning in the art, i.e., the genetic material of a virus that has been modified to serve as a vector to deliver genetic materials into a cell of interest in recombinant technology. Applicant respectfully submits that after entry of the claim amendments, claims 5 and 6 are definite.

The Examiner asserts that claim 16 is incomplete, because it is unclear what method step is intended by the recitation of "producing estrogen receptor."

Claim 16 has been amended to recite "thereby producing the estrogen receptor." After entry of the claim amendment, "producing the estrogen receptor" is no longer a method step, but a desired result from the culturing step. Applicant respectfully submits that the amended claim 16 is complete and definite.

The Examiner asserts that claims 8, 9 and 10 are vague and indefinite because these claims are directed to transformants, but also recite method steps, and that it is unclear whether these claims are directed to products or methods. The Examiner further asserts that, for claims 8-12, it is unclear if Applicant intends isolated cells or cells transformed *in vivo*. Claims 11-14 are rejected because they depend from the rejected claim 8.

Claims 8 and 9 have been amended to recite that the transformant is "produced by introducing ... into a host cell, wherein the host cell is isolated or cultured *in vitro*." Claim 10 has been amended to recite the transformant of claim 8 "comprising the estrogen receptor gene in a chromosome of the host cell." Applicant respectfully submits that after entry of the claim

amendments, claims 8-12 are definite, thus claims 11-14 are also definite because they no longer depend from allegedly indefinite claim 8.

The Examiner asserts that claim 17 is vague and indefinite in reciting "partial nucleotide sequence," because the specification has not provides a clear definition of this term. Claim 18 is also rejected because it depends from the rejected claim 17.

Without acquiescing to the Examiner's reasoning, claim 17 is cancelled. The cancellation renders the above rejection to claims 17 and 18 moot.

Accordingly, reconsideration and withdrawal of all rejections of claims under 35 USC § 112, second paragraph, are respectfully requested.

Claim Rejections under 35 USC § 112, First Paragraph

Claims 17 and 18 are rejected under 35 USC § 112, first paragraph, as failing to comply with the enablement requirement.

Without acquiescing to the Examiner's reasoning, claim 17 is cancelled. The cancellation renders the above rejection to claim 17 moot.

Regarding claim 18, the Examiner asserts that the specification does not teach any limiting structure, rather it teaches one exemplary sequence, and that art of record does not make up for the deficiencies in the specification in specifically delineating which nucleotides encode the estrogen (ligand) binding domain (LBD). Applicant respectfully disagrees.

The specification describes the LBD in sufficient detail which enables a person skilled in the art to make and use the LBD. In addition to the description about LBD as pointed out by the Examiner in the Office Action, the specification also provides specific examples on how to make and use a LBD from both an estrogen receptor alpha claimed in the present claims (Example 5, page 74, at line 23, to page 75, line 7), and a LBD of an estrogen receptor beta claimed in the present claims (Example 16, page 106, line 13, to page 108, line 3). Given the high degree of sequence similarities between estrogen receptor alpha (SEQ ID NO:1) and estrogen receptor alpha 2 (SEQ ID NO:4), one skilled in the art would readily know how to make and use the LBD of estrogen receptor alpha 2 based on the teaching of the present application.

In addition, at the time of the invention, LBDs for many estrogen receptors have been identified and studied. Structural and sequence homologies were observed among the LBDs. Ligands for estrogen receptors were readily available. Various *in vivo* or *in vitro* techniques could be used for ligand binding assays to measure the binding of a ligand to a LBD. In fact,

several years before the present application was filed, in the June 1999 version of the SWISS-PROT/TrEMBL database, there were already about 30 estrogen receptor LBD sequences from different species, omitting those with only partially determined sequences (see Mueller-Fahrnow et al., *Current Opinion in Biotechnology*, 1999, 10:550-556, hereinafter "1999 review paper," which accompanies this Amendment for reference). Crystal structures for a number of estrogen receptors had already been determined then. According to the 1999 review paper, the interspecies homology for the LBD is much greater than the similarity between the overall sequences of alpha and beta receptor subtypes (see page 550, 2nd column, 3rd paragraph). At the time of the invention, the state of the art was such that once the sequence of an estrogen receptor is known, one of ordinary skill in the art would readily predict the LBD of the estrogen receptor.

Claim 18 is directed to the LBD of an estrogen receptor gene of claim 1, which includes the estrogen receptor alpha and alpha 2 genes, and their related sequences. In view of the teaching of the present application, particularly in view of the sequences for the estrogen receptor alpha and alpha 2 genes provided in the present application, e.g., SEQ ID NOs:1 and 4 for the amino acid sequences, and SEQ ID NOs: 2 and 5 for the coding sequences, sequence alignment of the present estrogen receptor alpha and alpha 2 genes with other known estrogen receptor genes can be readily performed. The LBD sequences can be readily predicted based on the sequence alignment. Proteins containing the predicted LBD sequences can be readily made by DNA recombinant technology. The LBD functions of such proteins can be readily tested in *vivo* or *in vitro*. Therefore, a partial nucleotide sequence that encodes for a LBD of an estrogen receptor of the present application is enabled.

Accordingly, reconsideration and withdrawal of the rejections of claims 17 and 18 under 35 USC § 112, first paragraph, as failing to comply with the enablement requirement, are respectfully requested.

CONCLUSION

Reconsideration and withdrawal of all of the objections and rejections are respectfully solicited.

Applicant believes that the present application is now in condition for allowance. Upon finding composition claims allowable, Applicant respectfully requests the Examiner to rejoin

method claims that include all of the limitations of the allowable composition claims. A Notice of Allowance with respect to all elected composition claims and the rejoined method claims is respectfully solicited.

The Examiner is invited to contact the undersigned attorney for any reason to advance the prosecution of this application.

Respectfully submitted,

KOICHI SAITO

October 26, 2007 By: Alan S. Nadel
(Date)

ALAN S. NADEL
Registration No. 27,363
AKIN GUMP STRAUSS HAUER & FELD LLP
One Commerce Square
2005 Market Street, Suite 2200
Philadelphia, PA 19103-7013
Telephone: 215-965-1200
Direct Dial: 215-965-1280
Facsimile: 215-965-1210
E-Mail: anadel@akingump.com

ASN/WH/hg
Enclosures

United States Patent & Trademark Office

Appendix 1

10/501,227 **Estrogen receptor genes and utilization thereof**

Select New Case | Application Data | Transaction History | Image File Wrapper | Continuity Data | Foreign Priority | Published Documents | Address & Attorney/Agent | Supplemental Content

Supplemental Content - Search Results

This page gives you information about the number of versions associated with the application you request
Use this page to obtain specific version information.

Prev

Version Number	Item ID	Item Size
1	09323b67800d7a2e	147.879
1	09323b67800d7a31	147.879
1	09323b67800d7a34	147.879
1	09323b67800d7a37	147.879
1	09323b67800d7a3a	147.879
1	09323b67800d7a3e	147.879
1	09323b67800d7a41	147.879
1	09323b67800d7a44	147.879
1	09323b67800d7a47	147.879
1	09323b67800d7a4a	147.879
1	09323b67800d7a4d	147.879
1	09323b67800d7a50	147.879
1	09323b67800d7a54	147.879
1	09323b67800d7a57	147.879
1	09323b67800d7a5a	147.879
1	09323b67800d7a5d	147.879
1	09323b67800d7a61	147.879
1	09323b67800d7a64	147.879
1	09323b67800d9046	147.879
1	09323b67800d9049	147.879
1	09323b67800d904e	147.879
1	09323b67800d9051	147.879
1	09323b67800d9056	147.879
1	09323b67800d905b	147.879
1	09323b67800d9065	147.879
1	09323b67800d9067	147.879
1	09323b67800d906c	147.879
1	09323b67800d906f	147.879
1	09323b67800d9072	147.879
1	09323b67800d9075	147.879
1	09323b67800d9079	147.879
1	09323b67800d907c	147.879
1	09323b67800d9082	147.879
1	09323b67800d9a72	147.879
1	09323b67800d9a77	147.879
1	09323b67800d9a7c	147.879

10/501,227 **Estrogen receptor genes and utilization thereof**

GenCore version 6.2

OM protein - protein search, using sw model

Run on: March 10, 2007, 02:20:32 ; Search time 50 Seconds
(without alignments)
1887.708 Million cell updates/sec

Title: US-10-501-227-1
Perfect score: 2672
Sequence: 1 MSLKDWLLGKERTVVTMEEL..........PTGPGVLQYGGSRSDCTHIL 506

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 800936 seqs, 185091930 residues

Total number of hits satisfying chosen parameters: 800936

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Published_Applications_AA_New:*
1: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US08_NEW_PUB.pep:*
2: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US06_NEW_PUB.pep:*
3: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US07_NEW_PUB.pep:*
4: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/PCT_NEW_PUB.pep:*
5: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US09_NEW_PUB.pep:*
6: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US10_NEW_PUB.pep:*
7: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US11_NEW_PUB.pep:*
8: /EMC_Celerra_SIDS3/ptodata/2/pubpaa/US60_NEW_PUB.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	2672	100.0	506	6	US-10-501-227-1	Sequence 1, Appli
⇨ 2	2571	96.2	582	6	US-10-501-227-4	Sequence 4, Appli
3	2469	92.4	627	7	US-11-355-544-31	Sequence 31, Appl
4	2469	92.4	627	7	US-11-368-891-21	Sequence 21, Appl
5	2269.5	84.9	581	7	US-11-355-544-23	Sequence 23, Appl
6	2269.5	84.9	581	7	US-11-368-891-13	Sequence 13, Appl
7	2206.5	82.6	579	7	US-11-355-544-36	Sequence 36, Appl
8	2206.5	82.6	579	7	US-11-368-891-26	Sequence 26, Appl
9	2187.5	81.9	583	7	US-11-355-544-17	Sequence 17, Appl
10	2187.5	81.9	583	7	US-11-368-891-7	Sequence 7, Appli
11	2117.5	79.2	574	7	US-11-355-544-28	Sequence 28, Appl
12	2117.5	79.2	574	7	US-11-368-891-18	Sequence 18, Appl
13	2100.5	78.6	578	7	US-11-355-544-35	Sequence 35, Appl
14	2100.5	78.6	578	7	US-11-368-891-25	Sequence 25, Appl
15	2077.5	77.8	582	7	US-11-355-544-19	Sequence 19, Appl
16	2077.5	77.8	582	7	US-11-368-891-9	Sequence 9, Appli
17	2072	77.5	585	7	US-11-355-544-38	Sequence 38, Appl
18	2072	77.5	585	7	US-11-368-891-28	Sequence 28, Appl
19	1985	74.3	573	7	US-11-355-544-27	Sequence 27, Appl
20	1985	74.3	573	7	US-11-368-891-17	Sequence 17, Appl
21	1949.5	73.0	458	7	US-11-355-544-29	Sequence 29, Appl
22	1949.5	73.0	458	7	US-11-368-891-19	Sequence 19, Appl

23	1780	66.6	620	7	US-11-355-544-34	Sequence 34, Appl		
24	1780	66.6	620	7	US-11-368-891-24	Sequence 24, Appl		
25	1617	60.5	569	7	US-11-355-544-25	Sequence 25, Appl		
26	1617	2100.5	78.6	578	7	US-11-355-544-35	Sequence 35, Appl	
14	2100.5	78.6	578	7	US-11-368-891-25	Sequence 25, Appl		
15	2077.5	77.8	582	60.5	569	7	US-11-368-891-15	Sequence 15, Appl
27	1542.5	57.7	581	7	US-11-355-544-30	Sequence 30, Appl		
28	1542.5	57.7	581	7	US-11-368-891-20	Sequence 20, Appl		
29	1323	49.5	596	7	US-11-355-544-20	Sequence 20, Appl		
30	1323	49.5	596	7	US-11-368-891-10	Sequence 10, Appl		
31	1321.5	49.5	643	7	US-11-355-544-33	Sequence 33, Appl		
32	1321.5	49.5	643	7	US-11-368-891-23	Sequence 23, Appl		
33	1310	49.0	587	7	US-11-355-544-18	Sequence 18, Appl		
34	1310	49.0	587	7	US-11-368-891-8	Sequence 8, Appli		
35	1307.5	48.9	587	7	US-11-355-544-21	Sequence 21, Appl		
36	1307.5	48.9	587	7	US-11-368-891-11	Sequence 11, Appl		
37	1304	48.8	587	7	US-11-355-544-37	Sequence 37, Appl		
38	1304	48.8	587	7	US-11-368-891-27	Sequence 27, Appl		
39	1303	48.8	594	7	US-11-355-544-26	Sequence 26, Appl		
40	1303	48.8	594	7	US-11-368-891-16	Sequence 16, Appl		
41	1302.5	48.7	589	7	US-11-355-544-24	Sequence 24, Appl		
42	1302.5	48.7	589	7	US-11-368-891-14	Sequence 14, Appl		
43	1299.5	48.6	599	7	US-11-355-544-32	Sequence 32, Appl		
44	1299.5	48.6	599	7	US-11-368-891-22	Sequence 22, Appl		
45	1296	48.5	595	6	US-10-827-121-12	Sequence 12, Appl		

ALIGNMENTS

```
RESULT 1
US-10-501-227-1
; Sequence 1, Application US/10501227
; Publication No. US20060141560A1
; GENERAL INFORMATION:
;  APPLICANT: SAITO, Koichi
;  TITLE OF INVENTION: Estrogen receptor genes and utilization thereof
;  FILE REFERENCE: 600630-21US (561334)
;  CURRENT APPLICATION NUMBER: US/10/501,227
;  CURRENT FILING DATE:  2004-07-12
;  PRIOR APPLICATION NUMBER: JP 2002-004395
;  PRIOR FILING DATE: 2002-01-11
;  NUMBER OF SEQ ID NOS: 48
; SEQ ID NO 1
;   LENGTH: 506
;   TYPE: PRT
;   ORGANISM: Blue Gill
US-10-501-227-1

  Query Match              100.0%;  Score 2672;  DB 6;  Length 506;
  Best Local Similarity    100.0%;  Pred. No. 4.4e-215;
  Matches  506;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 MSLKDWLLGKERTVVTMEELRSSVPSSQQPVPREDQCATSDESYSVGESGAGARGFEMAK 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MSLKDWLLGKERTVVTMEELRSSVPSSQQPVPREDQCATSDESYSVGESGAGARGFEMAK 60

Qy         61 EMRFCAVCSDYASGYHYGVWSCEGCKAFFKRSIQGHNDYMCPATNQCTIDRNRRKSCQAC 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 EMRFCAVCSDYASGYHYGVWSCEGCKAFFKRSIQGHNDYMCPATNQCTIDRNRRKSCQAC 120

Qy        121 RLRKCYEVGMMKGGVRKDRGRVLRRDKRRAGTNDREKASKDLEYKTVPPQDRRKHSSSSS 180
              |||||||||||||||||||||||||||||||||27
;  CURRENT FILING DATE:  2004-07-12
;  PRIOR APPLICATION NUMBER: JP 2002-004395
;  PRIOR FILING DATE: 2002-01-11
;  NUMBER OF SEQ ID NOS: 48
; SEQ ID|||||||||||||||||||||||||||
Db        121 RLRKCYEVGMMKGGVRKDRGRVLRRDKRRAGTNDREKASKDLEYKTVPPQDRRKHSSSSS 180

Qy        181 AGGGGGKSSVTGMSPDQVLLLLQGAEPPMLCSRQKLSRPYTEVTIMTLLTSMADKELVHM 240
```

```
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 AGGGGGKSSVTGMSPDQVLLLLQGAEPPMLCSRQKLSRPYTEVTIMTLLTSMADKELVHM 240

Qy        241 ITWAKKLPGFLQLSLHDQVQLLESSWLEVLMIGLIWRSIHCPGKLIFAQDLILDRNEGDC 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 ITWAKKLPGFLQLSLHDQVQLLESSWLEVLMIGLIWRSIHCPGKLIFAQDLILDRNEGDC 300

Qy        301 VEGFVEIFDMLLATASRFRMLKLKPEEFVCLKAIILLNSGAFSFCTGTMEPLHNSMAVQN 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 VEGFVEIFDMLLATASRFRMLKLKPEEFVCLKAIILLNSGAFSFCTGTMEPLHNSMAVQN 360

Qy        361 MLDTITDALIHHISQSGCSAQQQSRRQAQLLLLLSHIRHMSNKGMEHLYSMKCKNKVPLY 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 MLDTITDALIHHISQSGCSAQQQSRRQAQLLLLLSHIRHMSNKGMEHLYSMKCKNKVPLY 420

Qy        421 DLLLEMLDAHRIHRPDRPAQFWSQADGEPPFINNNNSSNSGSNGGVSSSVGSSSGPRVNH 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        421 DLLLEMLDAHRIHRPDRPAQFWSQADGEPPFINNNNSSNSGSNGGVSSSVGSSSGPRVNH 480

Qy        481 ESPSRGPTGPGVLQYGGSRSDCTHIL 506
              ||||||||||||||||||||||||||
Db        481 ESPSRGPTGPGVLQYGGSRSDCTHIL 506

RESULT 2
US-10-501-227-4
; Sequence 4, Applicatio||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 ITWAKKLPGFLQLSLHDQVQLLESSWLEVLMIGLIWRSIHCPGKLIFAQDLILDRNEGDC 300

Qy        301 VEGFVEIFDMLLATASRFRMLKLKPEEFVCLKAIILLNSGAFSFCTGTMEPLHNSMAVQN 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 VEGFVEIFDMLLATASRFRMLKLKPEEFVCLKAIILLNSGAFSFCTGTMEPLHNSMAVQN 360

Qy        361 MLDTITDALIHHISQSGCSAQQQSRRQAQLLLLLSHIRHMSNKGMEHLYSMKCKNKVPLY 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 MLDTITDALIHHISQSGCSAQQQSRRQAQLLLLLSHIRHMSNKGMEn US/10501227
; Publication No. US20060141560A1
; GENERAL INFORMATION:
;  APPLICANT: SAITO, Koichi
;  TITLE OF INVENTION: Estrogen receptor genes and utilization thereof
;  FILE REFERENCE: 600630-21US (561334)
;  CURRENT APPLICATION NUMBER: US/10/501,227
;  CURRENT FILING DATE:  2004-07-12
;  PRIOR APPLICATION NUMBER: JP 2002-004395
;  PRIOR FILING DATE: 2002-01-11
;  NUMBER OF SEQ ID NOS: 48
; SEQ ID NO 4
;   LENGTH: 582
;   TYPE: PRT
;   ORGANISM: Blue Gill
US-10-501-227-4

  Query Match              96.2%;  Score 2571;  DB 6;  Length 582;
  Best Local Similarity   100.0%;  Pred. No. 1.5e-206;
  Matches  486;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy         21 RSSVPSSQQPVPREDQCATSDESYSVGESGAGARGFEMAKEMRFCAVCSDYASGYHYGVW 80
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         97 RSSVPSSQQPVPREDQCATSDESYSVGESGAGARGFEMAKEMRFCAVCSDYASGYHYGVW 156

Qy         81 SCEGCKAFFKRSIQGHNDYMCPATNQCTIDRNRRKSCQACRLRKCYEVGMMKGGVRKDRG 140
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        157 SCEGCKAFFKRSIQGHNDYMCPATNQCTIDRNRRKSCQACRLRKCYEVGMMKGGVRKDRG 216

Qy        141 RVLRRDKRRAGTNDREKASKDLEYKTVPPQDRRKHSSSSSAGGGGGKSSVTGMSPDQVLL 200
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        217 RVLRRDKRRAGTNDREKASKDLEYKTVPPQDRRKHSSSSSAGGGGGKSSVTGMSPDQVLL 276

Qy        201 LLQGAEPPMLCSRQKLSRPYTEVTIMTLLTSMADKELVHMITWAKKLPGFLQLSLHDQVQ 2PRT
;   ORGANISM: Blue Gill
US-10-501-227-4
```

```
  Query Match             96.2%;  Score 2571;  DB 6;  Length 582;
  Best Local Similarity   100.0%;  Pred. No. 1.5e-206;
  Matches  486;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy         21 RSSVPSSQQPVPREDQCATSDESYSVGESGAGARGFEMAKEMRFCAVCSDYASGYHYGVW 80
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         97 RSSVPSSQQPVPREDQCATSDESYSVGESGAGARGFEMAKEMRFCAVCSDYASGYHYGVW 156

Qy         81 SCEGCKAFFKRSIQGHNDYMCPATNQCTIDRNRRKSCQACRLRKCYEVGMMKGGVRKDRG 140
              ||||||||||||||60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        277 LLQGAEPPMLCSRQKLSRPYTEVTIMTLLTSMADKELVHMITWAKKLPGFLQLSLHDQVQ 336

Qy        261 LLESSWLEVLMIGLIWRSIHCPGKLIFAQDLILDRNEGDCVEGFVEIFDMLLATASRFRM 320
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        337 LLESSWLEVLMIGLIWRSIHCPGKLIFAQDLILDRNEGDCVEGFVEIFDMLLATASRFRM 396

Qy        321 LKLKPEEFVCLKAIILLNSGAFSFCTGTMEPLHNSMAVQNMLDTITDALIHHISQSGCSA 380
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        397 LKLKPEEFVCLKAIILLNSGAFSFCTGTMEPLHNSMAVQNMLDTITDALIHHISQSGCSA 456

Qy        381 QQQSRRQAQLLLLLSHIRHMSNKGMEHLYSMKCKNKVPLYDLLLEMLDAHRIHRPDRPAQ 440
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        457 QQQSRRQAQLLLLLSHIRHMSNKGMEHLYSMKCKNKVPLYDLLLEMLDAHRIHRPDRPAQ 516

Qy        441 FWSQADGEPPFINNNNSSNSGSNGGVSSSVGSSSGPRVNHESPSRGPTGPGVLQYGGSRS 500
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        517 FWSQADGEPPFINNNNSSNSGSNGGVSSSVGSSSGPRVNHESPSRGPTGPGVLQYGGSRS 576

Qy        501 DCTHIL 506
              ||||||
Db        577 DCTHIL 582


RESULT 3
US-11-355-544-31
; Sequence 31, Application US/11355544
; Publication No. US20060183159A1
; GENERAL INFORMATION:
;  APPLICANT: Zhao, Huimin
;  APPLICANT:  Chen, Zhilei
;  TITLE OF INVENTION: METHOD FOR ENGINEERING A PROTEIN BY IN VITRO COEVOLUTION
;  FILE REFERENCE: IL0013US
;  CURREPLHNSMAVQNMLDTITDALIHHISQSGCSA 380
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        397 LKLKPEEFVCLKAIILLNSGAFSFCTGTMEPLHNSMAVQNMLDTITDALIHHISQSGCSA 456

Qy        381 QQQSRRQAQLLLLLSHIRHMSNKGMEHLYSMKCKNKVPLYDLLLEMLDAHRIHRPDRPAQ 440
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        457 QQQSRRQAQLLLLLSHIRHMSNKGMEHLYSMKCKNKVPLYDLLLEMLDAHRIHRPDRPAQ 516

Qy        441 FWSQADGEPPFINNNNSSNSGSNGGVSSSVGSSSGPRVNHESPSRGPTGPGVLQYGGSRS 500
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db            NT APPLICATION NUMBER: US/11/355,544
;  CURRENT FILING DATE:  2006-02-16
;  PRIOR APPLICATION NUMBER: US 60/654,269
;  PRIOR FILING DATE: 2005-02-17
;  NUMBER OF SEQ ID NOS: 46
;  SOFTWARE: PatentIn version 3.3
; SEQ ID NO 31
;   LENGTH: 627
;   TYPE: PRT
;   ORGANISM: Micropterus salmoides
US-11-355-544-31

  Query Match             92.4%;  Score 2469;  DB 7;  Length 627;
  Best Local Similarity   96.9%;  Pred. No. 5.7e-198;
  Matches  471;  Conservative    4;  Mismatches    9;  Indels    2;  Gaps    2;

Qy         21 RSSVPSSQQPVPREDQCATSDESYSVGESGAGARGFEMAKEMRFCAVCSDYASGYHYGVW 80
```

Appendix 2

10/501,227 **Estrogen receptor genes and utilization thereof**

Select New Case | Application Data | Transaction History | Image File Wrapper | Continuity Data | Foreign Priority | Published Documents | Address & Attorney/Agent | Suppleme Conter

Supplemental Content - Search Results

This page gives you information about the number of versions associated with the application you request
Use this page to obtain specific version information.

Prev

Version Number	Item ID	Item Size	Start Download
1	09323b67800d7a2e	147.879	○
1	09323b67800d7a31	147.879	○
1	09323b67800d7a34	147.879	○
1	09323b67800d7a37	147.879	○
1	09323b67800d7a3a	147.879	○
1	09323b67800d7a3e	147.879	○
1	09323b67800d7a41	147.879	○
1	09323b67800d7a44	147.879	○
1	09323b67800d7a47	147.879	○
1	09323b67800d7a4a	147.879	○
1	09323b67800d7a4d	147.879	○
1	09323b67800d7a50	147.879	○
1	09323b67800d7a54	147.879	○
1	09323b67800d7a57	147.879	○
1	09323b67800d7a5a	147.879	○
1	09323b67800d7a5d	147.879	○
1	09323b67800d7a61	147.879	○
1	09323b67800d7a64	147.879	○
1	09323b67800d9046	147.879	○
1	09323b67800d9049	147.879	○
1	09323b67800d904e	147.879	○
1	09323b67800d9051	147.879	○
1	09323b67800d9056	147.879	○
1	09323b67800d905b	147.879	○
1	09323b67800d9065	147.879	○
1	09323b67800d9067	147.879	○
1	09323b67800d906c	147.879	○
1	09323b67800d906f	147.879	○
1	09323b67800d9072	147.879	○
1	09323b67800d9075	147.879	○
1	09323b67800d9079	147.879	○
1	09323b67800d907c	147.879	○
1	09323b67800d9082	147.879	◉
1	09323b67800d9a72	147.879	○
1	09323b67800d9a77	147.879	○
1	09323b67800d9a7c	147.879	○

10/501,227 **Estrogen receptor genes and utilization thereof**

```
GenCore version 6.2
            Copyright (c) 1993 - 2007  Biocceleration Ltd.


OM nucleic - nucleic search, using sw model

Run on:         March 11, 2007, 15:24:15 ; Search time 1219 Seconds
                                           (without alignments)
                                           8479.387 Million cell updates/sec

Title:          US-10-501-227-5_COPY_74_1819
Perfect score:  1746
Sequence:       1 atgtaccctgaagagagcag.........ctgactgcacccacatccta 1746

Scoring table:  IDENTITY_NUC
                Gapop 10.0 , Gapext 1.0

Searched:       11113607 seqs, 2959867085 residues

Total number of hits satisfying chosen parameters:      22227214

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
                 Maximum Match 100%
                 Listing first 45 summaries

Database :       Published_Applications_NA_New:*
                1:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US08_NEW_PUB.seq:*
                2:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US06_NEW_PUB.seq:*
                3:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US07_NEW_PUB.seq:*
                4:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/PCT_NEW_PUB.seq:*
                5:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US09_NEW_PUB.seq:*
                6:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq:*
                7:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq1:*
                8:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq2:*
                9:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq3:*
                10:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq4:*
                11:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq5:*
                12:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq6:*
                13:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_NEW_PUB.seq:*
                14:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_NEW_PUB.seq1:*
                15:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_NEW_PUB.seq2:*
                16:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_NEW_PUB.seq3:*
                17:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_NEW_PUB.seq4:*
                18:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US60_NEW_PUB.seq:*

    Pred. No. is the number of results predicted by chance to have a
    score greater than or equal to the score of the result being printed,
    and is der0_NEW_PUB.seq1:*
                8:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq2:*
                9:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq3:*
                10:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq4:*
                11:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq5:*
                12:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US10_NEW_PUB.seq6:*
                13:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_NEW_PUB.seq:*
                14:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_NEW_PUB.seq1:*
                15:  /EMC_Celerra_SIDS3/ptodata/2/pubpna/US11_ived by analysis of the tota

                                SUMMARIES
                   %
 Result          Query
   No.   Score   Match Length DB  ID                    Description
 ---------------------------------------------------------------------------
     1    1746   100.0   1824  11  US-10-501-227-5       Sequence 5, Appli
 ⇨  2    1674    95.9   3499  11  US-10-501-227-2       Sequence 2, Appli
     3   988.6    56.6    996  11  US-10-501-227-3       Sequence 3, Appli
     4   482.6    27.6   1788  16  US-11-355-544-1       Sequence 1, Appli
```

	5	482.6	27.6	1788	16	US-11-368-891-1 Sequence 1, Appli
	6	481	27.5	1788	16	US-11-355-544-15 Sequence 15, Appl
	7	481	27.5	6450	8	US-10-940-774-18 Sequence 18, Appl
	8	481	27.5	6450	11	US-10-515-675A-47 Sequence 47, Appl
	9	481	27.5	6450	11	US-10-517-155A-25 Sequence 25, Appl
	10	481	27.5	6450	14	US-11-289-102-120 Sequence 120, App
	11	481	27.5	6450	14	US-11-283-329-91 Sequence 91, Appl
	12	481	27.5	6450	15	US-11-346-759-100 Sequence 100, App
	13	479.4	27.5	1788	16	US-11-355-544-7 Sequence 7, Appli
	14	477.8	27.4	1788	16	US-11-355-544-9 Sequence 9, Appli
	15	476.2	27.3	1788	16	US-11-355-544-11 Sequen 2, Appli
	3	988.6	56.6	996	11	US-10-501-227-3 Sequence 3, Appli
	4	482.6	27.6	1788	16	US-11-355-544-1 Sequence 1, Appli
	5	482.6	27.6	1788	16	US-11-368-891-1 Sequence 1, Appli
	6	481	27.5	1788	16	US-11-355-544-15 Sequence 15, Appl
	7	481	27.5	6450	8	US-10-940-774-18 Sequence 18, Appl
	8	481	27.5	6450	11	US-10-515-675A-47 Sequence 47, Appl
	9	481	27.5	6450	11	US-10-517-155A-25 Sequence 25, Appl
	10	481	27.5	64ce	11,	Appl
	16	474.6	27.2	1788	16	US-11-355-544-13 Sequence 13, Appl
	17	428.4	24.5	2186	11	US-10-501-227-24 Sequence 24, Appl
	18	389.8	22.3	1593	16	US-11-355-544-5 Sequence 5, Appli
	19	389.8	22.3	1593	16	US-11-368-891-5 Sequence 5, Appli
	20	389.8	22.3	1745	8	US-10-940-774-1563 Sequence 1563, Ap
	21	389.8	22.3	2011	14	US-11-283-329-93 Sequence 93, Appl
	22	389.8	22.3	2011	15	US-11-346-759-101 Sequence 101, App
	23	361	20.7	882	16	US-11-355-544-3 Sequence 3, Appli
	24	361	20.7	882	16	US-11-368-891-3 Sequence 3, Appli
	25	353	20.2	6039	11	US-10-541-683-6 Sequence 6, Appli
	26	353	20.2	14411	11	US-10-541-683-7 Sequence 7, Appli
	27	346.2	19.8	2739	16	US-11-280-456-39 Sequence 39, Appl
c	28	346.2	19.8	2739	16	US-11-280-456-44 Sequence 44, Appl
	29	346.2	19.8	2775	16	US-11-280-456-41 Sequence 41, Appl
c	30	346.2	19.8	2775	16	US-11-280-456-43 Sequence 43, Appl
	31	340.8	19.5	963	11	US-10-501-227-25 Sequence 25, Appl
	32	299.6	17.2	1000	14	US-11-266-748A-395231 Sequence 395231,.
c	33	299.6	17.2	1000	14	US-11-266-748A-466277 Sequence 466277,
	34	292	16.7	5001	8	US-10-940-774-1495 Sequence 1495, Ap
	35	240	13.7	553	11	US-10-834-268-2626 Sequence 2626, Ap
	36	234.4	13.4	751	11	US-10-956-160-3191 Sequence 3191, Ap
	37	230	13.2	529	11	US-10-834-268-468 Sequence 468, App
	38	215	12.3	611	14	US-11-266-748A-214650 Sequence 214650,
	39	191.8	11.0	600	11	US-10-956-160-213298 Sequence 213298,
	40	173.4	9.9	600	11	US-10-956-160-213297 Sequence 213297,
	41	173.4	9.9	748	11	US-10-956-160-3190 Sequence 3190, Ap
	42	133.6	7.7	1503	11	US-10-526-940-7 Sequence 7, Appli
	43	132.6	7.6	1566	11	US-10-526-940-2 Sequence 2, Appli
	44	132.6	7.6	2402	14	US-11-266-748A-32133 Sequence 32133, A
	45	132	7.6	2193	14	US-11-283-329-97 Sequence 97, Appl

ALIGNMENTS

RESULT 1
US-10-501-227-5
; Sequence 5, Application US/10501227
; Publication No. US20060141560A1
; GENERAL INFORMATION:
; APPLICANT: SAITO, Koichi
; TITLE OF INVENTION: Estrogen receptor genes and utilization thereof
; FILE REFERENCE: 600630-21US (561334)
; CURRENT APPLICATION NUMBER: US/10/501,227
; CURRENT FILING DATE: 2004-07-12
; PRIOR APPLICATION NUMBER: JP 2002-004395
; PRIOR FILING DATE: 2002-01-11
; NUMBER OF SEQ ID NOS: 48
; SEQ ID NO 5
; LENGTH: 1824
; TYPE: DNA 213298,
40 173.4 9.9 600 11 US-10-956-160-213297 Sequence 213297,

```
    41   173.4    9.9    748   11  US-10-956-160-3190           Se
;   ORGANISM: Blue Gill
;   FEATURE:
;   NAME/KEY: CDS
;   LOCATION: (74)...(1822)
US-10-501-227-5

  Query Match              100.0%;  Score 1746;  DB 11;  Length 1824;
  Best Local Similarity    100.0%;  Pred. No. 0;
  Matches 1746;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 ATGTACCCTGAAGAGAGCAGGGGGTCCGGAGGGGTAGCCACTGTGGACTTTCTGGAAGGG 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         74 ATGTACCCTGAAGAGAGCAGGGGGTCCGGAGGGGTAGCCACTGTGGACTTTCTGGAAGGG 133

Qy         61 ACCTACGATTATGCCGCCCCCACCCCTGCCCCGACTCCTCTTTACAGCCAGTCTGGCTAC 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        134 ACCTACGATTATGCCGCCCCCACCCCTGCCCCGACTCCTCTTTACAGCCAGTCTGGCTAC 193

Qy        121 TACTCTGTACCTCTGGACGCCCAAGGGCCACCCTCAGATGGCAGCCTTCAGTCCCTGGGC 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        194 TACTCTGTACCTCTGGACGCCCAAGGGCCACCCTCAGATGGCAGCCTTCAGTCCCTGGGC 253

Qy        181 AGCGGGCCTACCAGTCCTCTTGTGTTTGTGCCGTCCAGCCCCAGACTCAGCCCCTTTATG 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        254 AGCGGGCCTACCAGTCCTCTTGTGTTTGTGCCGTCCAGCCCCAGACTCAGCCCCTTTATG 313

Qy        241 CACCCGCCCAGCCACCACTATCTGGAAACCACCTCAACACCCGTCTACAGGTCTAGTGTC 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        314 CACCCGCCCAGCCACCACTATCTGGAAACCACCTCAACACCCGTCTACAGGTCTAGT|||||||||||||||
Db         74 ATGTACCCTGAAGAGAGCAGGGGGTCCGGAGGGGTAGCCACTGTGGACTTTCTGGAAGGG 133

Qy         61 ACCTACGATTATGCCGCCCCCACCCCTGCCCCGACTCCTCTTTACAGCCAGTCTGGCTAC 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        134 ACCTACGATTATGCCGCCCCCACCCCTGCCCCGACTCCTCTTTACAGCCAGTCTGGCTAC 193

Qy        121 TACTCTGTACCTCTGGACGCCCAAGGGCCACCCTCAGATGGCAGCCTTCAGTCCCTGGGC 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        194 TACTCTGTACCTCTGGACGCCCAAGGGCCACCCTCAGATGGCAGCCTTCAGTCCCTGGGC 253

Qy        181 AGCGGGCCTGTC 373

Qy        301 CCATCCAGCCAGCAGCCAGTTCCCAGAGAGGACCAGTGTGCCACCAGTGATGAGTCCTAT 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        374 CCATCCAGCCAGCAGCCAGTTCCCAGAGAGGACCAGTGTGCCACCAGTGATGAGTCCTAT 433

Qy        361 AGTGTGGGGGAGTCAGGGGCTGGAGCCAGGGGGTTTGAGATGGCCAAGGAGATGCGTTTC 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        434 AGTGTGGGGGAGTCAGGGGCTGGAGCCAGGGGGTTTGAGATGGCCAAGGAGATGCGTTTC 493

Qy        421 TGTGCTGTGTGCAGTGACTATGCCTCTGGGTACCACTACGGGGTGTGGTCCTGTGAAGGC 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        494 TGTGCTGTGTGCAGTGACTATGCCTCTGGGTACCACTACGGGGTGTGGTCCTGTGAAGGC 553

Qy        481 TGTAAGGCCTTCTTTAAGAGGAGCATCCAGGGTCACAATGACTATATGTGCCCAGCAACC 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        554 TGTAAGGCCTTCTTTAAGAGGAGCATCCAGGGTCACAATGACTATATGTGCCCAGCAACC 613

Qy        541 AATCAGTGTACTATTGACAGGAATCGGAGAAAGAGCTGCCAGGCTTGCCGTCTTAGGAAG 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        614 AATCAGTGTACTATTGACAGGAATCGGAGAAAGAGCTGCCAGGCTTGCCGTCTTAGGAAG 673

Qy        601 TGTTATGAAGTGGGCATGATGAAAGGAGGTGTTCGCAAGGACCGTGGCCGTGTTTTGCGC 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        674 TGTTATGAAGTGGGCATGATGAAAGGAGGTGTTCGCAAGGACCGTGGCCGTGTTTTGCGC 733

Qy        661 CGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGTAC 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        734 CGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGTAC 793

Qy        721 AAAACAGTGCCCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGCAGTGCCGGTGGTGGA 780
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      794 AAAACAGTGCCCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGCAGTGCCGGTGGTGGA 853

Qy      781 GGAGGAAAATCATCAGTGACCGGGATGTCTCCTGACCAGGTGCTCCTCCTGCTCCAGGGT 840
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      854 GGAGGAAAATCATCAGTGACCGGGATGTCTCCTGACCAGGTGCTCCTCCTGCTCCAGGGT 913

Qy      841 GCCGAGCCCCCAATGCTGTGCTCCCGTCAGAAGCTGAGCCGACCGTACACCGAGGTCACC 900
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      914 GCCGAGCCCCCAATGCTGTGCTCCCGTCAGAAGCTGAGCCGACCGTACACCGAGGTCACC 973

Qy      901 ATAATGACACTACTCACCAGCATGGCCGATAAGGAGCTGGTCCACATGATCACCTGGGCC 960
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      974 ATAATGACACTACTCACCAGCATGGCCGATAAGGAGCTGGTCCACATGATCACCTGGGCC 1033

Qy      961 AAGAAGCTTCCAGGTTTCCTGCAGCTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGAGC 1020
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1034 AAGAAGCTTCCAGGTTTCCTGCAGCTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGAGC 1093

Qy     1021 TCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCAAA 1080
            |||||GAGGAAAATCATCAGTGACCGGGATGTCTCCTGACCAGGTGCTCCTCCTGCTCCAGGGT 840
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      854|||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1094 TCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCAAA 1153

Qy     1081 CTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCTTT 1140
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1154 CTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCTTT 1213

Qy     1141 GTTGAGATCTTCGACATGCTGCTGGCCACTGCCTCCCGCTTCCGCATGCTCAAACTCAAA 1200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1214 GTTGAGATCTTCGACATGCTGCTGGCCACTGCCTCCCGCTTCCGCATGCTCAAACTCAAA 1273

Qy     1201 CCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCTTCTCTTTC 1260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1274 CCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCTTCTCTTTC 1333

Qy     1261 TGCACCGGCACAATGGAGCCCCTCCACAACAGCATGGCAGTGCAGAACATGCTGGACACC 1320
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1334 TGCACCGGCACAATGGAGCCCCTCCACAACAGCATGGCAGTGCAGAACATGCTGGACACC 1393

Qy     1321 ATCACAGACGCTCTCATACATCATATCAGCCAATCAGGATGCTCGGCTCAGCAGCAGTCG 1380
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1394 ATCACAGACGCTCTCATACATCATATCAGCCAATCAGGATGCTCGGCTCAGCAGCAGTCG 1453

Qy     1381 AGGCGGCAGGCCCAGCTGCTGCTCCTGCTCTCCCACATCAGGCACATGAGCAACAAAGGC 1440
            ||||||||||||||||||||||||||||||||||||||||||||||||||CAAACTCAAA 1200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1214 GTTGAGATCTTCGACATGCTGCTGGCCACTGCCTCCCGCTTCCGCATGCTCAAACTCAAA 1273

Qy     1201 CCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCTTCTCTTTC 1260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1274 CCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCTTCTCTTTC 1333

Qy     1261 TGCACCGGCACAATGGAGCCCCTCCACAACAGCATGGCAGTGCAGAACATGCTGGACACC 1320
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1334 TGCACCGGCAC|||||||||
Db     1454 AGGCGGCAGGCCCAGCTGCTGCTCCTGCTCTCCCACATCAGGCACATGAGCAACAAAGGC 1513

Qy     1441 ATGGAGCATCTCTACAGCATGAAGTGCAAGAACAAAGTGCCTCTTTACGACCTTCTGCTG 1500
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1514 ATGGAGCATCTCTACAGCATGAAGTGCAAGAACAAAGTGCCTCTTTACGACCTTCTGCTG 1573

Qy     1501 GAGATGTTGGACGCTCACCGTATACACCGCCCAGACAGACCAGCTCAGTTCTGGTCCCAG 1560
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1574 GAGATGTTGGACGCTCACCGTATACACCGCCCAGACAGACCAGCTCAGTTCTGGTCCCAG 1633

Qy     1561 GCTGACGGAGAGCCTCCCTTCATTAACAACAACAACAGCAGCAACAGTGGCAGCAATGGC 1620
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1634 GCTGACGGAGAGCCTCCCTTCATTAACAACAACAACAGCAGCAACAGTGGCAGCAATGGC 1693
```

```
Qy       1621 GGCGTCTCCTCTTCAGTCGGTTCCAGTTCAGGACCCCGAGTCAACCACGAGAGCCCGAGC 1680
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1694 GGCGTCTCCTCTTCAGTCGGTTCCAGTTCAGGACCCCGAGTCAACCACGAGAGCCCGAGC 1753

Qy       1681 AGAGGACCCACAGGTCCAGGAGTCCTGCAGTACGGAGGGTCCCGCTCTGACTGCACCCAC 1740
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1754 AGAGGACCCACAGGTCCAGGAGTCCTGCAGTACGGAGGGTCCCGCTCTGACTGCACCCAC 1813

Qy       1741 ATCCTA 1746
              ||||||
Db       1814 ATCCTA 1819
```

```
RESULT 2
US-10-501-227-2
; Sequence 2, Application US/10501227
; Publication No. US20060141560A1
; GENERAL INFORM||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1574 GAGATGTTGGACGCTCACCGTATACACCGCCCAGACAGACCAGCTCAGTTCTGGTCCCAG 1633

Qy       1561 GCTGACGGAGAGCCTCCCTTCATTAACAACAACAACAGCAGCAACAGTGGCAGCAATGGC 1620
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1634 GCTGACGGAGAGCCTCCCTTCATTAACAACAACAACAGCAGCAACAGTGGCAGCAATGGC 1693

Qy       1621 GGCGTCTCCTCTTCAGTCGGTTCCAGTTCAGGACCCCGAGTCAACCACGAGAGCCCGAGC 1680
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1694 GGCGTCTCCTCTTCAGTCGGTTCCAGTTCAGGACCCCGAGTCAACCACGAGAGCCATION:
;  APPLICANT: SAITO, Koichi
;  TITLE OF INVENTION: Estrogen receptor genes and utilization thereof
;  FILE REFERENCE: 600630-21US (561334)
;  CURRENT APPLICATION NUMBER: US/10/501,227
;  CURRENT FILING DATE:  2004-07-12
;  PRIOR APPLICATION NUMBER: JP 2002-004395
;  PRIOR FILING DATE: 2002-01-11
;  NUMBER OF SEQ ID NOS: 48
; SEQ ID NO 2
;   LENGTH: 3499
;   TYPE: DNA
;   ORGANISM: Blue Gill
;   FEATURE:
;   NAME/KEY: CDS
;   LOCATION: (424)...(1944)
US-10-501-227-2

  Query Match             95.9%;  Score 1674;  DB 11;  Length 3499;
  Best Local Similarity   96.6%;  Pred. No. 0;
  Matches 1746;  Conservative    0;  Mismatches    0;  Indels   62;  Gaps    1;

Qy          1 ATGTACCCTGAAGAGAGCAGGGGGTCCGGAGGGGTAGCCACTGTGGACTTTCTGGAAGGG 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        134 ATGTACCCTGAAGAGAGCAGGGGGTCCGGAGGGGTAGCCACTGTGGACTTTCTGGAAGGG 193

Qy         61 ACCTACGATTATGCCGCCCCCACCCCTGCCCCGACTCCTCTTTACAGCCAGTCTGGCTAC 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        194 ACCTACGATTATGCCGCCCCCACCCCTGCCCCGACTCCTCTTTACAGCCAGTCTGGCTAC 253

Qy        121 TACTCTGTACCTCTGGACGCCCAAGGGCCACCCTCAGATGGCAGCCTTCAGTCCCTGGGC 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        254 TACTCTGTACCTCTGGACGCCCAAGGGCCACCCTCAGATGGCAGCCTTCAGTCCCTGGGC 313

Qy        181 AGCGGGCCTACCAGTCCTCTTGTGTTTGTGCCGTCCAGCCCCAGACTCAGCCCCTTTATG 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        314 AGCGGGCCTACCAGTCCTCTTGTGTTTGTGCCGTCCAGCCCCAGACTCAGCCCCTTTATG 373

Qy        241 CACCCGCCCAGCCACCACTATCTGGAAACCACCTCAACACCCGTCTAC------------ 288
              ||||||||||||||||||||||||||||||||||||||||||||||||
Db        374 CACCCGCCCAGCCACCACTATCTGGAAACCACCTCAACACCCGTCTACAGATGAGTCTGA 433

Qy        289 --------------------------------------------------AGGTCTAGTG 298
                                                                ||||||||||
Db        434 AAGACTGGTTATTAGGAAAAGAAAGGACGGTGGTGACCATGGAGGAGCTGAGGTCTAGTG 493
```

```
Qy     299 TCCCATCCAGCCAGCAGCCAGTTCCCAGAGAGGACCAGTGTGCCACCAGTGATGAGTCCT 358
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     494 TCCCATCCAGCCAGCAGCCAGTTCCCAGAGAGGACCAGTGTGCCACCAGTGATGAGTCCT 553

Qy     359 ATAGTGTGGGGGAGTCAGGGGCTGGAGCCAGGGGGTTTGAGATGGCCAAGGAGATGCGTT 418
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     554 ATAGTGTGGGGGAGTCAGGGGCTGGAGCCAGGGGGTTTGAGATGGCCAAGGAGATGCGTT 613

Qy     419 TCTGTGCTGTGTGCAGTGACTATGCCTCTGGGTACCACTACGGGGTGTGGTCCTGTGAAG 478
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     614 TCTGTGCTGTGTGCAGTGACTATGCCTCTGGGTACCACTACGGGGTGTGGTCCTGTGAAG 673

Qy     479 GCTGTAAGGCCTTCTTTAAGAGGAGCATCCAGGGTCACAATGACTATATGTGCCCAGCAA 538
           ||||||||||||||||||||||||||||||||||||||||||||||------------AGGTCTAGTG 298
                                                                 ||||||||||
Db     434 AAGACTGGTTATTAGGAAAAGAAAGGACGGTGGTGA||||||||||||
Db     674 GCTGTAAGGCCTTCTTTAAGAGGAGCATCCAGGGTCACAATGACTATATGTGCCCAGCAA 733

Qy     539 CCAATCAGTGTACTATTGACAGGAATCGGAGAAAGAGCTGCCAGGCTTGCCGTCTTAGGA 598
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     734 CCAATCAGTGTACTATTGACAGGAATCGGAGAAAGAGCTGCCAGGCTTGCCGTCTTAGGA 793

Qy     599 AGTGTTATGAAGTGGGCATGATGAAAGGAGGTGTTCGCAAGGACCGTGGCCGTGTTTTGC 658
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     794 AGTGTTATGAAGTGGGCATGATGAAAGGAGGTGTTCGCAAGGACCGTGGCCGTGTTTTGC 853

Qy     659 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 718
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     854 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 913

Qy     719 ACAAAACAGTGCCCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGCAGTGCCGGTGGTG 778
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     914 ACAAAACAGTGCCCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGCAGTGCCGGTGGTG 973

Qy     779 GAGGAGGAAAATCATCAGTGACCGGGATGTCTCCTGACCAGGTGCTCCTCCTGCTCCAGG 838
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     974 GAGGAGGAAAATCATCAGTGACCGGGATGTCTCCTGACCAGGTGCTCCTCCTGCTCCAGG 1033

Qy     839 GTGCCGAGCCCCCAATGCTGTGCTCCCGTCAGAAGCTGAGCCGACCGTACACCGAGGTCA 898
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    1034 GTGCCGAGCCCCCAATGCTGTGCTCCC||||||||||||||||||||||||||||||||||||||||||||||
Db     794 AGTGTTATGAAGTGGGCATGATGAAAGGAGGTGTTCGCAAGGACCGTGGCCGTGTTTTGC 853

Qy     659 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 718
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     854 GCCGTGATAAACGACGTGCTGGAACCAATGACCGAGAGAAGGCCTCTAAGGACCTGGAGT 913

Qy     719 ACAAAACAGTGCCCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGCAGTGCCGGTGGTG 778
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     914 ACAAAACAGTGCCCCCTCAGGACAGGAGGAAACACAGCAGCAGCAGCAGTGCCGGTGGTGGTCAGAAGCTGAGCCG

Qy     899 CCATAATGACACTACTCACCAGCATGGCCGATAAGGAGCTGGTCCACATGATCACCTGGG 958
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    1094 CCATAATGACACTACTCACCAGCATGGCCGATAAGGAGCTGGTCCACATGATCACCTGGG 1153

Qy     959 CCAAGAAGCTTCCAGGTTTCCTGCAGCTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGA 1018
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    1154 CCAAGAAGCTTCCAGGTTTCCTGCAGCTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGA 1213

Qy    1019 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCA 1078
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    1214 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCA 1273

Qy    1079 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1138
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    1274 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1333

Qy    1139 TTGTTGAGATCTTCGACATGCTGCTGGCCACTGCCTCCCGCTTCCGCATGCTCAAACTCA 1198
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db    1334 TTGTTGAGATCTTCGACATGCTGCTGGCCACTGCCTCCCGCTTCCGCATGCTCAAACTCA 1393
```

```
Qy     1199 AACCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCTTCTCTT 1258
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1394 AACCTGAGGAGTTTGTCTGCCTCAAAGCTATCATCCTGCTCAACTCTGGTGCCTTCTCTT 1453

Qy     1154 CCAAGAAGCTTCCAGGTTTCCTGCAGCTGTCTCTCCATGACCAGGTGCAGCTGCTGGAGA 1213

Qy     1019 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCA 1078
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1214 GCTCGTGGCTGGAGGTGCTGATGATTGGGCTCATATGGAGGTCCATCCACTGCCCCGGCA 1273

Qy     1079 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1138
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1274 AACTCATCTTCGCACAGGACCTCATACTGGACAGGAATGAAGGTGACTGTGTGGAAGGCT 1333

Qy     1139 TTGTTGAGATCTTCGACATGCTGCTGG    1259 TCTGCACCGGCACAATGGAGCCCCTCCACAACAGCATGGC
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1454 TCTGCACCGGCACAATGGAGCCCCTCCACAACAGCATGGCAGTGCAGAACATGCTGGACA 1513

Qy     1319 CCATCACAGACGCTCTCATACATCATATCAGCCAATCAGGATGCTCGGCTCAGCAGCAGT 1378
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1514 CCATCACAGACGCTCTCATACATCATATCAGCCAATCAGGATGCTCGGCTCAGCAGCAGT 1573

Qy     1379 CGAGGCGGCAGGCCCAGCTGCTGCTCCTGCTCTCCCACATCAGGCACATGAGCAACAAAG 1438
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1574 CGAGGCGGCAGGCCCAGCTGCTGCTCCTGCTCTCCCACATCAGGCACATGAGCAACAAAG 1633

Qy     1439 GCATGGAGCATCTCTACAGCATGAAGTGCAAGAACAAAGTGCCTCTTTACGACCTTCTGC 1498
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1634 GCATGGAGCATCTCTACAGCATGAAGTGCAAGAACAAAGTGCCTCTTTACGACCTTCTGC 1693

Qy     1499 TGGAGATGTTGGACGCTCACCGTATACACCGCCCAGACAGACCAGCTCAGTTCTGGTCCC 1558
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1694 TGGAGATGTTGGACGCTCACCGTATACACCGCCCAGACAGACCAGCTCAGTTCTGGTCCC 1753

Qy     1559 AGGCTGACGGAGAGCCTCCCTTCATTAACAACAACAACAGCAGCAACAGTGGCAGCAATG 1618
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1754 AGGCTGACGGAGAGCCTCCCTTCATTAACAACAACAACAGCAGCAACAGTGGCAGCAATG 1813

Qy     1619 GCGGCGTCTCCTCTTCAGTCGGTTCCAGTTCAGGACAGGATGCTCGGCTCAGCAGCAGT 1573

Qy     1379 CGAGGCGGCAGGCCCAGCTGCTGCTCCTGCTCTCCCACATCAGGCACATGAGCAACAAAG 1438
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1574 CGAGGCGGCAGGCCCAGCTGCTGCTCCTGCTCTCCCACATCAGGCACATGAGCAACAAAG 1633

Qy     1439 GCATGGAGCATCTCTACAGCATGAAGTGCAAGAACAAAGTGCCTCTTTACGACCTTCTGC 1498
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1634 GCATGGAGCATCTCTACAGCATGAAGTGCAAGAACAAAGTGCCTCTTTACGACCTTCTGC 1693

Qy     1499 TGGAGATGTTGGACGCTCACCGTATACACCGCCCAGACAGACCAGCTCAGTTCTGGTCCC 1558
       CCCCGAGTCAACCACGAGAGCCCGA 1678
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1814 GCGGCGTCTCCTCTTCAGTCGGTTCCAGTTCAGGACCCCGAGTCAACCACGAGAGCCCGA 1873

Qy     1679 GCAGAGGACCCACAGGTCCAGGAGTCCTGCAGTACGGAGGGTCCCGCTCTGACTGCACCC 1738
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db     1874 GCAGAGGACCCACAGGTCCAGGAGTCCTGCAGTACGGAGGGTCCCGCTCTGACTGCACCC 1933

Qy     1739 ACATCCTA 1746
            ||||||||
Db     1934 ACATCCTA 1941
```

RESULT 3
US-10-501-227-3
; Sequence 3, Application US/10501227
; Publication No. US20060141560A1
; GENERAL INFORMATION:
; APPLICANT: SAITO, Koichi
; TITLE OF INVENTION: Estrogen receptor genes and utilization thereof
; FILE REFERENCE: 600630-21US (561334)

Ligand-binding domain of estrogen receptors

Anke Mueller-Fahrnow* and Ursula Egner†

Estrogen receptors are multi-domain proteins that interact with other proteins and DNA to fulfil their function: the regulation of transcription. During the past 2–3 years, our understanding of this complex process has increased tremendously as crystal structures of isolated ligand-binding domains in complex with various ligands, as well as co-activator peptides, are now available. The structural information, combined with new data on novel co-activators/co-repressors, muteins and their actions, and novel ligands, allows for the first time the development of detailed theories for the first steps of transcription initiation.

Addresses
Research Laboratories of Schering AG, D-13342 Berlin, Germany
*e-mail: anke.muellerfahrnow@schering.de
†e-mail: ursula.egner@schering.de

Current Opinion in Biotechnology 1999, 10:550–556

Abbreviations

DES	diethylstilbestrol
E2	estradiol
ER	estrogen receptor
h	human
LBD	ligand-binding domain
LBP	ligand-binding pocket
NR	nuclear receptor
RXR	retinoic X receptor

Introduction

The estrogen receptor (ER) belongs to the superfamily of nuclear receptors (NRs). It regulates the expression of specific genes and thus plays a crucial role in many processes, such as the control of reproduction and the development of secondary sexual characteristics [1,2]. It has therefore been a target for research within pharmaceutical companies for many years. As a result, thousands of steroidal and non-steroidal ligands to the ER have been synthesized, which act either as agonists, partial agonists or pure antagonists.

NRs are built up by five to six domains serving different functions. Out of these, the ligand-binding domain (LBD; E-domain) is of special interest not only due to its involvement in ligand binding but also in receptor dimerization and in the transactivation process: the carboxy-terminal region of the LBD comprises the transactivation function AF-2 [3•]. Until recently, the structural basis for these functions has only been poorly understood, but during the past few years crystal structures for the human (h)ERα LBD [4,5,6••] and hERβ LBD [7], as well as for a number of other NR LBDs, have been determined [8–15]. With these structures at hand, the differences and similarities between hERα LBDs (Egner U *et al.*, unpublished data) and the recently identified hERβ [7,16] can be analyzed. Although the availability of the structures has contributed to our understanding of ER actions, a number of open questions remain, especially concerning the conformational changes that occur upon ligand binding (described below). In this review, we give an overview of new data on estrogen receptor LBDs with a special emphasis on structural aspects. We try to outline the implications of the structure on our understanding of the protein function and point out areas for future research.

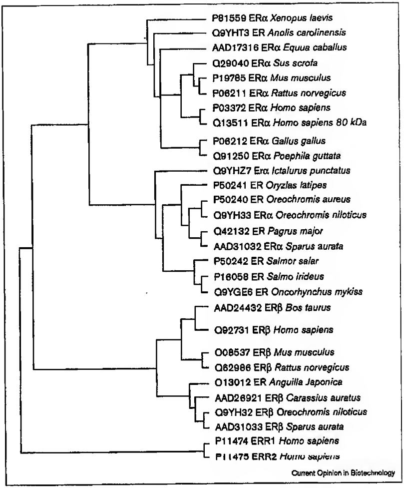
Homology with other nuclear receptors

With respect to sequence homology, the superfamiliy of NRs can be divided into several subfamilies. The ER subfamily comprises ERα and ERβ, which share ~55% sequence identity in their LBDs (two proteins are expected to share a similar fold if the sequence identity is >30%, and if the identity is as high as 50% the three-dimensional structures can be assumed to be very similar), and the estrogen-receptor-related orphan receptors ERR1 and ERR2 [17]. On the mRNA level, different splice variants have been identified for ERs, but the corresponding proteins have not been detected [18–23].

In the June 1999 version of the SWISS-PROT/TrEMBL database [24], around 30 ER LBD sequences from different species were found, omitting those with only partially determined sequences. Based on a multiple sequence alignment, a phylogenetic tree was reconstructed (Figure 1). From this presentation, it is obvious that the interspecies homology for the LBDs is much higher than the similarity between the α and β receptor subtypes. It should also be mentioned that for both ER homologues, LBDs from fish and vertebrates can be clearly distinguished with respect to their sequence homologies (Figure 1).

The distribution of conserved residues on the surface of the hERα LBD is shown in Figure 2a. Highly conserved residues within the ER subfamily are indicated by yellow colouring of the protein, which is shown in the same orientation as in Figure 3. A striking feature of the ER subfamily is that most of the conserved residues within the LBD are located in one area of the protein, close to one assumed co-activator binding site (see below). A similar analysis for the progesterone receptor subfamily is given in Figure 2b. The human proteins belonging to this subgroup (progesterone, glucocorticoid, androgen and mineralocorticoid receptors) share ~50% sequence identity within their LBDs, but conserved residues are more equally distributed (Figure 2b). The relevance of this observation is not clear. We know, however, that LBDs function via conformational changes that occur upon ligand binding and by interactions with other ER domains and with co-activators/co-repressors. It can therefore be speculated that a highly conserved region

Figure 1



Phylogenetic reconstruction of the ER LBD sequences (amino acid range as shown in Figure 4) according to the neighbor-joining method as implemented in CLUSTALW V1.7 [49]. Accession numbers referring to the Swiss-PROT/TrEMBL database [24] are given. The ERR1 and ERR2 sequences are defined as outgroup.

on the protein surface is indicative of a conserved site for protein–protein interactions.

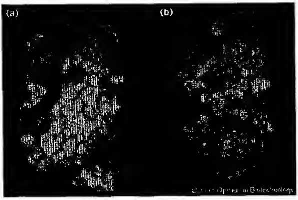
The three-dimensional structure

For most steroid receptors, as well as for a number of related nuclear receptors, crystal structures of LBDs have been determined during the past few years; however, not all of them have been published. In addition to the experimentally determined structures, a number of models have been published based on related structures and on a number of additional supporting biological and binding data [25,26•]. An overview of the ER LBD structures and on some relevant models is given in Table 1. The overall structures of the hERα and hERβ LBD–raloxifene complexes are very similar, the root-mean-square deviation between equivalent Cα atoms (excluding helix H12) is 0.97 Å [7].

All known NR LBD structures consist of 11–12 α-helices and one small β-sheet. The secondary structure elements are arranged in a so-called α-helical sandwich, a protein fold that has up to now only been observed for NR LBDs. This fold is shown as a cartoon in Figure 3 together with the numbering of some of the helices. Helix H2, which has been observed, for example, in the retinoic X receptor (RXR) LBD structure [8] is absent in the hERα LBD. The mainly hydrophobic binding niche, which is described below in more detail, is located in the lower part of the protein with respect to the orientation of the protein shown in Figure 3.

A detailed comparison with the structures of other NR LBDs shows that the upper part of the protein (with respect to the orientation shown in Figure 3) is structurally much better conserved than the lower part harbouring the ligand-binding pocket (LBP) (for details see [3•]; Egner U *et al.*, unpublished data). This can be rationalized as the natural ligands differ considerably in size and shape; the structural variability can thus be attributed to the affinity towards diverse ligands.

Figure 2



Connolly surface of the hERα LBD in complex with (a) estradiol compared to the (b) progesterone receptor LBD. The orientation of the proteins is the same as in Figure 3. Coloring is based on residue conservation: yellow, highly conserved residues (> 80% identity); blue, homologous residues (> 80% similarity); green, no sequence conservation. The red arrow indicates the position of helix H12, the tip of the arrow being the carboxy-terminus of the helix.

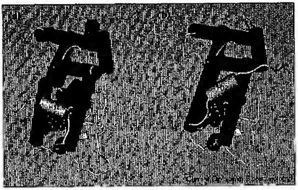
The ligand-binding pocket

ERs bind the endogenous ligand estradiol with a very high affinity of 0.05 nM for hERα and 0.09 nM for hERβ, respectively [27]. The protein–ligand interactions have been analyzed and described in detail [28–31,32•]. The otherwise hydrophobic LBP, which is not accessible from the protein surface in the agonist-bound state (see below), harbours two polar regions located at opposite ends of the cavity. At the deep end of the pocket, Glu353 and Arg394 (hERα numbering) serve as anchoring points for the 3-hydroxy group of estradiol (E2). In addition, a conserved water molecule stabilizes this interaction. This hydrogen bond network explains the predominant contribution of the 3-hydroxy group to the binding affinity of the steroid [25]. Similar hydrogen bonds are observed in all available hERα and hERβ LBD structures.

The 17-hydroxy group of E2 interacts with the second polar region within the LBP, that is, with His524 (hERα numbering). Different sidechain conformations for this residue are observed in the various complex structures known to date. Except for these two polar regions, the LBP is lined by hydrophobic residues. The fact that some are flexible (e.g. methionine and isoleucine) accounts for the high affinity of a variety of steroidal and non-steroidal ligands, including phytoestrogens such as genistein [7,27].

As the estrogen receptors are of such high medical relevance, thousands of ligands have been synthesized within research institutions and in the pharmaceutical industry. With respect to their functions, the ligands can be classified as agonists (e.g. E2 [32•]) partial agonists (e.g. raloxifene [28]) and as pure antagonists [30,32•]. Up to now, structures are only available for the first two groups, so that the structural basis

Figure 3



Cartoon [50] of the hERα LBD in complex with (a) the endogenous ligand estradiol and (b) the partial agonist raloxifene. The helices are represented as barrels. For some of them, the numbering is given.

for the differences between partial agonists and pure antagonists is thus not yet understood.

Conformational changes

For the hERα LBD, seven crystal structures are available. The most striking difference between these structures is the orientation of helix H12. A comparison with related structures ([4,6••]; Ruff M *et al.*, personal communication) indicates that the position of this helix depends on the presence of a ligand. In the only available *apo* form of a NR LBD, the RXR LBD, this helix points away from the protein core [8]. In most of the available agonist-bound LBDs, however, the helix is folded back and serves as a lid closing the entrance to the LBP. At the same time, AF-2 is brought into a position where co-activators can bind resulting in transcription initiation. In the case of the hERα LBD, helix H12 is anchored in this 'agonist conformation' by hydrophobic interactions as well as by hydrogen bonds.

The more space-consuming ER partial agonists with their long-chain substituents cannot be accommodated in the LBP in the 'agonist conformation' of the protein. It was therefore expected that upon partial agonist binding, helix H12 would be present in another orientation. The two different orientations of helix H12 induced by the agonist E2 and the partial agonist raloxifene are depicted in Figure 3, showing that this is the case. Recently, the crystal structure has been determined for the ternary complex of the hERα LBD with the agonist diethylstilbestrol (DES) and a peptide of the co-activator GRIP-1 [6••]. The position of the α-helical peptide almost coincides with helix H12 in the antagonist conformation of the protein: upon superposition, the distance between equivalent Cα positions varies between 0.6–2.2 Å. This observation supports the experimental finding that antagonists block co-activator binding.

In contrast to expectation, two structures of the hERα LBD in complex with E2 are presently known where helix

Figure 4

```
ERα mouse   312 LSLTADQMVA LLDAEPPMIY SEY DPSRPF SEASMMGLLT NLAD 355
ERβ human   208 DALSPEQLVL TLLEAEPPHV LIS.RPSAPF TEASMMMSLT KLAD 250
ERα human   308 LSLT[illegible]AEPPIL YSEYDPTRPF SE[illegible] 351
Mutations                                        x xxx     x
Helices              H1                          H3

ERα mouse   356 RELVHMINWA KRVPGFGDLN LHDQVHLLEC AWLEILMIGL VWRSMEHPG  403
ERβ human   251 KELVHMISWA KKIPGFVELS LFDQVRLLES CWMEVLMMGL MWRSIDHPG  299
ERα human   352 [illegible]RVPGFVDLT [illegible]EC A[illegible]SMEHPG  400
Mutations         x         x x   x     x x   xx  x x x         X   x
Helices                               H4 ·        H5

ERα mouse   404 KLLFAPNLLL DRNQGKCVEG MVEIFDMLLA TSSRFRMMNL QGEEFVCLKS 453
ERβ human   300 KLIFAPDLVL DRDEGKCVEG ILEIFDMLLA TTSRFRELKL QHKEYLCVKA 349
ERα human   401 [illegible]FAPNL[illegible]RNQGKCVEG [illegible]MNL Q[illegible] 450
Mutations                   X   xxx   x  X x     X                x
Helices                               H7                    H8

ERα mouse   454 IILLNSGVYT FLSSTLKSLE EKDHIHRVLD KITDTLIHLM AKAGLTLQQQ 503
ERβ human   350 MILLNSSMYP LVT.ATQDAD SSRKLAHLLN AVTDALVWVI AKSGISSQQQ 398
ERα human   451 [illegible]LNSGVYT FLSST[illegible]AGLT[illegible] 500
Helices                          H9                               H10

Mutations           x   x  x x  x       x   x  x x   x  xxxx  xxxx
ERα mouse   504 HRRLAQLLLI LSHIRHMSNK GMEHLYNMKC KNVVPLYDLL LEMLDAH RLH 553
ERβ human   399 SMRLANLLML LSHVRHASNK GMEHLLNMKC KNVVPVYDLL LEMLNAH VLR 448
ERα human   501 [illegible] KNVVPL[illegible]DAH RLH 550
Mutations           x   xxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx x  x   x
Helices             H10                 H11             H12
```

Current Opinion in Biotechnology

Sequence alignment of hERα, mouse ERα and hERβ LBDs. Positions of mutated residues are marked by an (x) below (hERα) or above (mouse ERα) the sequences. Residues belonging to helices in the hERα LBD–estradiol complex are indicated by a gray background; residues in contact with ligands are printed bold face; the numbers of the helices are given.

H12 is not in the agonist conformation. In the artificial tetramer crystallized by Tannenbaum *et al.* [5], this helix interacts with a neighboring LBD molecule, and in the recently crystallized triple mutant (Ruff M *et al.*, personal communication), the helix is in the 'partial agonist conformation'. The meaning of this structural variablity is not yet fully understood but might be caused by the absence of the carboxy-terminal F-domain (adjacent to helix H12) of the hER in all crystal structures. There is no experimental evidence that the structural variability observed in the structures is caused by the absence of the F-domain. It can not, however, be excluded that an additional domain has a stability effect on three dimensional structure.

Differences between receptor subtypes

The tissue distribution of hERα and hERβ was investigated shortly after the discovery of the second isoform [16]. hERβ is the predominant subtype in bone, blood vessels and in the brain, whereas its content in uterus and liver is only low. Because of the different distribution in the various target organs, the medical interest in subtype-specific ligands is high.

Both human receptors share ~55% identical amino acid residues in their LBDs. The analysis of the LBPs shows that most residues in direct contact with E2 in the hERα LBD are conserved in hERβ. The same is true for residues in the second shell around the ligand. There are a number of differences, however, in the vicinity of the steroidal A-ring close to position 4 of estradiol. In this area, six homologous replacements are encountered (e.g. Ile→Leu, Met or Val). Another two homologous exchanges are present in the environment of the steroidal D-ring. In spite of the high degree of conservation between the LBPs, an ERα subtype-specific ligand exhibiting a more than 100-fold difference in binding affinity has recently been published [31].

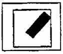
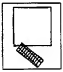
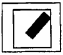
Dimerization and binding of co-activators/ co-repressors

Receptor dimerization is a prerequisite for transcription initiation in ER-regulated processes. As LBDs contribute to the dimerization interface, it was expected that the hERα–E2 complex would crystallize as a dimer, as is the case. There is evidence that the interactions observed in the crystals reflect the natural dimerization contacts: firstly, all published point mutants influencing ER dimerization are in, or at least close to, a region involved in crystal contact; and secondly, dimers have been observed in crystal structures of related LBDs ([4,6••]; Ruff M *et al.*, personal communication). The topology of the dimer interfaces is comparable in all these structures, especially concerning the key role of helix H10 in the intermolecular contacts. In the case of the hERα–E2 complex, the monomer–monomer interactions are very strong: 27 amino acid residues contribute to the large contact surface and out of these 14 are involved in hydrogen bonds.

In recent years, several novel classes of transcriptional mediators (co-activators and co-repressors) that interact

Table 1

Published crystal structures of hERα and hERβ LBDs.

LBD	Ligand/co-activator peptide			Resolution (Å)	Relative position of helix H12
	Endogenous ligand/agonist	Partial agonist/ antagonist	Co-activator peptide		
hERα LBD					
LBD carboxy-methylated [6••]	DES		GRIP-1: 686–698	2.0	
Wild-type LBD [(a)]	Estradiol			3.2	
LBD carboxy-methylated [4]	Estradiol			3.1	
Mutant LBD: C381S/C417S/ C530S [(a)]	Estradiol			2.2	
LBD carboxy-methylated [6••]		Tamoxifen		1.9	
LBD carboxy-methylated [4]		Raloxifene		2.6	
Tetramer due to intermolecular disulfide bond [5]	Estradiol			2.8	
Model [25]	Estradiol and others			Not applicable	
Model [26•]	Estradiol and others			Not applicable	
hERβ LBD [7]					
LBD carboxy-methylated		Genistein		1.8	
		Raloxifene		2.25	

(a) M Ruff, M Gangloff, M Eiler, S Duclaud, JM Wurtz, D Moras, unpublished data. DES, diethylstilbestrol.

with LBDs have been identified and characterized [33–35,36•,37–39]. It should be mentioned here that some of these mediators, such as NSD1 and GRIP-1, are ligand dependent [33,36•], whereas for others, such as SRC-1, a hormone-independent mechanism is discussed [39]. The recently elucidated structure of hERα LBD in complex with a GRIP-1 peptide has already been mentioned above [6••]; however, a comparable complex structure with a hormone-independent mediator is not yet available.

Mutations

In contrast to, for example, the androgen receptor, only one germline mutation is known for the hER, which led to osteoporosis in a young man [40]. A number of splice variants have been identified on the mRNA level and their biological role remains to be elucidated. Furthermore, some point mutations have been identified in patients with primary breast cancer [41].

For a better understanding of the functional role of different residues, a number of engineered mutants of the hERα LBD have been investigated ([26•] and references therein, [41–48]), but only four mutants for hERβ LBD have yet been published. Positions of mutated residues are indicated in the sequence alignment shown in Figure 4. For a number of these mutants, the influence on ligand binding has been analyzed in detail (e.g. [43], and references in [26•]). For the vast majority of them the influence on the binding activity could be understood on the basis of the hERα LBD structure. In the more recent literature, the effect of muteins on the transcription potency and on the interaction with co-activators/co-repressors is also described (e.g. [44,46]).

Conclusions

During the past few years, the progress in our understanding of the hERα LBD actions has been tremendous. Several scientific areas have contributed to this achievement. Firstly, after more than one decade of intensive work, crystal structures of hERα and hERβ LBD complexes, as well as of related NR LBDs, are now available. Secondly, novel classes of co-activators and co-repressors have been identified and characterized, which have been mentioned only briefly in this review. Thirdly, new steroidal and non-steroidal ligands to the hERs have been synthesized. A special emphasis has been on the search for pure antagonists, which are considered to have an

improved clinical profile as compared to the commonly used partial agonists, such as tamoxifen. Finally, the effect of engineered mutants has been studied in detail and supported our understanding of the role of single residues within the protein subunit.

In spite of the progress outlined above, many open questions and uncertaincies remain concerning the ER LBD actions. To give one example, no crystal structure is available for a complex with a pure antagonist, that is, the structural basis for the functional differences between partial agonists and pure antagonists is unknown. Furthermore, the role of helix H12 and its different orientations has in part been deducted from crystal structures in which the domain adjacent to helix H12, the F-domain, is missing. The conformational variability of this helix might thus be an artefact depending upon the choice of the protein fragment used for the crystallization trials.

If the number of new ER LBD structures further increases at its current rate and if the information about the co-activators and co-repressors continues to contribute equally fast to our understanding of ER actions, we will soon be able to form a realistic picture on the early stages of transcriptional activation.

Acknowledgements

We thank N Heinrich, D Moras and J-M Wurtz for helpful and stimulating discussions and B Weiss for bioinformatic support. We are grateful to G-A Hoyer for continuous support.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Katzenellenbogen BS: **Estrogen receptors: bioactivities and interaction with cell signaling pathways.** *Biol Reprod* 1996, 54:287-293.

2. Ciocca DR, Roig LM: **Estrogen receptors in human nontarget tissues: biological and clinical implications.** *Endocrinol Rev* 1995, 16:35-62.

3. Moras D, Gronemeyer H: **The nuclear receptor ligand-binding**
• **domain: structure and function.** *Curr Opin Cell Biol* 1998, 10:384-391.
The authors give an overview of different nuclear receptor ligand-binding domains structures in a condensed form. The most relevant information on co-activators/co-repressors is also presented.

4. Brzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T, Engström O, Öhman L, Green GL, Gustafsson JA, Carlquist M: **Molecular basis of agonism and antagonism in the oestrogen receptor.** *Nature* 1997, 389:753-758.

5. Tannenbaum DM, Wang Y, Williams SP, Sigler PB: **Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains.** *Proc Natl Acad Sci USA* 1998, 95:5998-6003.

6. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA,
•• Greene GL: **The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen.** *Cell* 1998, 95:927-937.
The first example of a crystal structure of a ternary complex of the hERα ligand-binding domain (LBD) in complex with an agonist (diethylstilbestrol) and a co-activator fragment. The crystal structure of the hER LBD in complex with a partial agoniet, tamoxifen, is also presented. The structural basis for co-activator recognition is also discussed with respect to the interaction of tamoxifen with the protein.

7. Pike ACW, Brzozowski AM, Hubbard RE, Bonn I, Thorsell AG, Engström O, Ljunggren J, Gunstafsson JA, Corlquist M: **Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full agonist.** *EMBO J* 1999, 18:4608-4618.

8. Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D: **Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha.** *Nature* 1995, 375:377-382.

9. Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, Moras D: **Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid.** *Nature* 1995, 378:681-689.

10. Wagner RL, Apriletti JW, McGralh ME, West BL, Baxter JD, Fletterick RJ: **A structural role for hormone in the thyroid hormone receptor.** *Nature* 1995, 378:690-697.

11. Ribeiro RCJ, Apriletti JW, Wagner RL, Feng W, Kushner PJ, Nilsson S, Scanlan TS, West BL, Fletterick RJ, Baxter JD: **X-ray crystallographic and functional studies of thyroid hormone receptor.** *J Steroid Biochem Molec Biol* 1998, 65:133-141.

12. Williams SP, Sigler PB: **Atomic structure of progesterone complexed with its receptor.** *Nature* 1998, 393:392-396.

13. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV: **Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ.** *Nature* 1998, 395:137-143.

14. Uppenberg J, Svensson C, Jaki M, Bertilsson G, Jendeberg L, Berkenstam A: **Crystal structure of the ligand binding domain of the human nuclear receptor PPARγ.** *J Biol Chem* 1998, 273:31108-31112.

15. Klaholz BP, Renaud JP, Mitschler A, Zusi C, Chambon P, Gronemeyer H, Moras D: **Conformational adaption of agonists to the human nuclear receptor RARγ.** *Nat Struct Biol* 1998, 5:199-202.

16. Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA: **Cloning of a novel estrogen receptor expressed in rat prostate and ovary.** *Proc Natl Acad Sci USA* 1996, 93:5925-5930.

17. Giguere V, Yang N, Segui P, Evans RM: **Identification of a new class of steroid hormone receptors.** Nature 1988, 331:91-94.

18. Saunders P: **Oestrogen receptor beta (ERβ).** *Rev Reprod* 1998, 3:164-171.

19. Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL, Su JL, Kliewer SA, Lehmann JM, Willson TM: **Cloning and characterization of human estrogen receptor β isoforms.** *Biochem Biophys Res Commun* 1998, 247:75-78.

20. Vladusik EA, Hornby AE, Guerra Vladusik FK, Lupu R: **Espression of estrogen receptor beta messenger RNA variant in breast cancer.** *Cancer Res* 1998, 58:210-214.

21. Petersen DN, Tkalcevic GT, Koza-Taylor PH, Turi TG, Brown TA: **Identification of estrogen receptor β2, a functional variant of estrogen receptor β expressed in normal rat tissues.** *Endocrinology* 1998, 139:1082-1092.

22. Maruyama K, Endoh H, Sasaki-Iwaoka H, Kanou H, Shimaya E, Hashimoto S, Kato S, Kawashima H: **A novel isoform of rat estrogen receptor beta with 18 amino acid insertion in the ligand binding domain as a putative dominant negative regulator of estrogen action.** *Biochem Biophys Res Commun* 1998, 246:142-147.

23. Murphy LC, Dotzlaw H, Leygue E, Douglas D, Coutts A, Watson PH: **Estrogen receptor variants and mutations.** *J Steroid Biochem Mol Biol* 1997, 62:363-372.

24. Bairoch A, Apweiler R: **The SWISS-PROT protein sequence data bank and its supplement TrEMBL in 1999.** *Nucleic Acid Res* 1999, 27:49-54.

25. Anstead GM, Carlson KE, Katzenellenbogen JA: **The estradiol pharmacophore: ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site.** *Steroids* 1997, 62:268-303.

26. Wurtz JM, Egner U, Heinrich N, Moras D, Mueller-Fahrnow A: **Three**
• **dimensional models of estrogen receptor ligand binding domain complexes, based on related crystal structures and mutational and structure-activity relationship data.** *J Med Chem* 1998, 11:1803-1814.
Thorough comparative modelling study. The hERα ligand-binding domain (LBD) model is evaluated based on the analysis of ligands and their relative binding affinities. The article provides a list of mutants within the LBD of human and mouse estrogen receptor evaluating the binding affinities with respect to the wild-type receptor.

27. Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA: **Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β.** *Endocrinology* 1998, 139:4252-4263.

28. Grese TA, Dodge JA: **Selective estrogen receptor modulators (SERMs).** *Curr Pharmaceut Des* 1998, 4:71-92.

29. Levenson AS, Jordan VC: **The key to antiestrogenic mechanism of raloxifene is amino acid 351 (aspartate) in the estrogen receptor.** *Cancer Res* 1998, 58:1872-1875.

30. Macgregor JI, Jordan VC: **Basic guide to the mechanism of antiestrogen action.** *Pharmacol Rev* 1998, 50:151-196.

31. Sun J, Meyers MJ, Fink BE, Rajendran R, Katzenellenbogen JA, Katzenellenbogen BS: **Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-α or estrogen receptor-β.** *Endocrinology* 1999, 140:800-804.

32. Gao H, Katzenellenbogen JA, Garg R, Hansch C: **Comparative • QSAR analysis of estrogen receptor ligands.** *Chem Rev* 1999, 99:723-744.
This study provides detailed information on comparative structure-activity relationships of steroidal and non-steroidal estrogen receptor ligands. Factors influencing the relative binding affinity, for example, the steric and polar character of substituents are highlighted. The quality of the derived relationships is critically analysed.

33. Huang N, von Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P, Loeson R: **Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators.** *EMBO J* 1998, 17:3398-3412.

34. vom Baur E, Harbers M, Um SJ, Benecke A, Chambon P, Losson R: **The yeast Ada complex mediates the ligand-dependent activation function AF-2 of retinoid X and estrogen receptors.** *Genes Dev* 1998, 12:1278-1289.

35. Johansson L, Thomsen JS, Damdimopoulos AE, Spyrou G, Gustafsson JA, Treuter E: **The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activation of estrogen receptors ERα and ERβ.** *J Biol Chem* 1999, 274:345-353.

36. Norris JD, Fan D, Stallcup MR, McDonnell DP: **Enhancement of • estrogen receptor transcriptional activity by the coactivator GRIP-1 highlights the role of activation function 2 in determining estrogen receptor pharmacology.** *J Biol Chem* 1998, 273:6679-6688.
The authors analyze the interaction of the co-activator GRIP1 with the estrogen receptor. They suggest that GRIP1 may not only interact with the transactivation function 2 (AF-2) of the ligand-binding domain (LBD) but also contacts the receptor in yet unidentified areas of the LBD. The results are supported by mutagenesis experiments of selected amino acid residues in helix H12 of the LBD.

37. Peters GA, Khan SA: **Estrogen receptor domains E and F: role in dimerisation and interaction with coactivator RIP-140.** *Mol Endocrinol* 1999, 13:286-296

38. Parker MG: **Transcriptional activation by oestrogen receptors.** *Biochem Soc Symp* 1998, 63:45-50.

39. Smith CL, Nawaz Z, O'Malley BW: **Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen.** *Mol Endocrinol* 1997, 11:657-666.

40. Smith EP, Boyd J, Frank G, Takahashi H, Cohen R, Specker B, Williams T, Lubahn D, Korach K: **Estrogen resistance caused by a mutation in the estrogen receptor gene in a man.** *N Engl J Med* 1994, 331:1056-1061.

41. Umekita Y, Sagara Y, Yoshida H: **Estrogen receptor mutations and changes in estrogen receptor and progesterone receptor protein expression in metastatic or recurrent breast cancer.** *Jpn J Cancer Res* 1998, 89:27-32.

42. Nichols M, Rientjes JMJ, Stewart AF: **Different positioning of the ligand-binding domain helix 12 and the F domain of the estrogen receptor accounts for functional differences between agonists and antagonists.** *EMBO J* 1998, 17:765-773.

43. Ekena K, Katzenellenbogen JA, Katzenellenbogen BS: **Determinants of ligand binding specificity of estrogen receptor-α: estrogen versus androgen discrimination.** *J Biol Chem* 1998, 273:693-699.

44. Tremblay GB, Tremblay A, Labrie F, Giguère V: **Ligand-independent activation of the estrogen receptors α and β by mutations of a conserved tyrosine can be abolished by antiestrogens.** *Cancer Res* 1998, 58:877-881.

45. Tremblay GB, Trembley A, Labrie F, Giguère V: **Dominant activity of activation function 1 (AF-1) and differential stoichiometric requirements for AF-1 and -2 in the estrogen receptor α-β heterodimeric complex.** *Mol Cell Biol* 1999, 19:1919-1927.

46. Feng W, Ribeiro RCJ, Wagner RL, Nguyen H, Apriletti JW, Fletterick RJ, Baxter JD, Kushner PJ, West BL: **Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors.** *Science* 1998, 280:1747-1749.

47. Taylor JA, Lewis KJ, Lubahn DB: **Estrogen receptor mutations.** *Mol Cell Endocrinol* 1998, 145:61-66.

48. Miller N, Whelan J: **Random mutagenesis of human estrogen receptor ligand binding domain identifies mutations that decrease sensitivity to estradiol and increase sensitivity to a diphenol indene-ol compound: basis for a regulatable expression system.** *J Steroid Biochem Mol Biol* 1998, 64:129-135.

49. Thompson JD, Higgins DG, Gibson TJ: **CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice.** *Nucleic Acid Res* 1994, 22:4673-4680.

50. Insight98.0. San Diego, USA: MSI Inc.; 1998.